Amendments to the Specification:

Please replace paragraph 1, beginning at line 3, with the following amended paragraph:

The present invention relates to the preservation of biological and other labile samples, to such preserved samples and to a novel process for preserving such samples. The novel process comprises adding a sample including an active agent and a ~~stabilising~~ stabilizing agent to a container, subjecting the sample to such temperature and pressure conditions to cause solvent loss by evaporation without freezing the sample or bubbling to form a foam. Subsequently, during a secondary drying phase, pressure and temperature conditions are maintained or adjusted so that solvent is removed and the preservation sample dries to form a highly viscous liquid. Further provided by the present invention are compositions preserved by the process of the present invention and in particular preserved vaccine compositions.

Please replace paragraph 2, beginning at line 14, with the following amended paragraph:

There is a need to extend the stability and thus the shelf life of labile samples, particularly biological samples. Traditionally, this has been accomplished using the process of freeze drying in which a solution of the substance is made and the sample is frozen. During the primary drying phase, most of the water is removed by sublimation from ice under reduced pressure conditions and a porous 'cake' is formed. This is usually followed by a secondary drying phase when the pressure and temperature are changed and water is evaporated from the solid 'cake'. The resulting ~~lyophilised~~ lyophilized sample has improved stability compared to a liquid formulation. However, the freeze drying process is lengthy, expensive and can be the rate limiting step in a production process.

Please replace paragraph 3, beginning at line 31, with the following amended paragraph:

Damage caused by the process of freezing may be circumvented to some degree by the use of ~~stabilising~~ stabilizing agents such as polyols. Further improvements on the process of ~~lyophilisation~~ lyophilization have also been made by avoiding freezing the sample during the process and removing water by boiling (WO96/40077; US6306345). This method involves preparing a mixture of a glass-matrix forming material in a suitable solvent together with the sample to be preserved, evaporating bulk solvent from the mixture to obtain a syrup, exposing the syrup to a pressure and temperature sufficient to cause boiling of the syrup and removing residual solvent. Methods similar to this may be referred to as foam drying techniques. Such techniques will expose the sample to be preserved to stresses due to the formation and bursting of bubbles during the 'boiling' stage. Especially where labile substances are to be preserved, this may result in a loss of activity.

Please replace paragraph 3, on page 2, beginning at line 21, with the following amended paragraph:

Trehalose is a polyol that is favoured for its ~~stabilising~~ stabilizing properties. Trehalose is a naturally occurring, inert, non-reducing and non-toxic, glass-forming disaccharide that was initially found to be associated with the prevention of desiccation damage in some plants and animals. Trehalose is useful in preventing denaturation of a wide variety of substances including proteins, viruses and foodstuffs during desiccation and subsequent storage partly because it has a relatively high glass transition temperature (ca 120 °C in the anhydrous state) (US4891319; US5149653; US5026566). Trehalose also ~~stabilises~~ stabilizes enzymes (Argall and Smith (1993) Biochem. Mol. Biol. Int. 30; 491). Trehalose and a wide variety of ~~stabilising~~ stabilizing polyols have also been found to be useful in improving the preservation of freeze-dried samples, especially in cases where the sample is prone to loss of activity during the freeze-drying process. Other sugars useful in ~~lyophilisation~~ lyophilization techniques include sucrose and lactose.

Please replace paragraph 1, on page 3, with the following amended paragraph:

The present invention relates to an improved method of preserving an active agent, particularly if the active agent is labile and prone to loss of activity during a more

conventional drying process. The process comprises the steps of preparing a preservation sample by dissolving/suspending an active agent in a solution of a ~~stabilising~~ stabilizing agent; subjecting the preservation sample to such temperature and pressure conditions that the preservation sample looses solvent by evaporation, without the sample freezing or bubbling to form a foam; and removing solvent until the preservation sample dries to form a highly viscous liquid.

Please replace paragraph 5, on page 4, beginning at line 10, with the following amended paragraph:

The method of the invention is used for preserving an active agent and comprises the steps of:

a) [[•]] preparing a preservation sample by suspending or dissolving an active agent in a solution of a ~~stabilising~~ stabilizing agent;

b) [[•]] subjecting the preservation sample to such temperature and pressure conditions that the preservation sample looses solvent by evaporation, without freezing or bubbling to form a foam, to form a viscous liquid;

and optionally includes a further step of:

c) [[•]] removing solvent until the viscous liquid dries to form a highly viscous liquid.

Please replace paragraph 3, on page 5, beginning at line 9, with the following amended paragraph:

A viscous liquid is defined as the product of the primary phase of solvent removal, at the end of which the majority of solvent has been lost from the sample. This point can be ~~recognised~~ recognized because the rate of evaporation slows down so that the temperature of the sample returns to the ambient temperature as the endothermic effect of bulk evaporation is lost.

Please replace paragraph 4, on page 5, beginning at line 15, with the following amended paragraph:

A highly viscous liquid is produced after the viscous liquid produced at the end of the primary phase of drying has been exposed to reduced pressure for a further period of time after the end of the primary phase of drying. A highly viscous liquid has a solvent content less than or equal to 15, 12, 10, 8, 5, 4, 3, 2 or 1% (w/w), preferably as determined by Karl Fischer coulometric moisture ~~analyser~~ analyzer (Eur. J. Pharm. Biopharm. (2000) 50; 277-284). Preferred ranges of solvent content are 1-3%, 3-5%, 5-10% or 10-15% (w/w). The highly viscous liquid has a sufficiently low solvent content such that the active agent is preserved in a stable state for at least 3, 6, 9,12 or 24 months at 4 °C, allowing the active agent to retain at least 40, 50, 60, preferably 70, 80, 90, 95% of its activity and/or antigenicity and/or immunogenicity over this period. Preferably, the highly viscous liquid has a solid appearance but is a rubber or glass, preferably a glass and is able to flow very slowly over a period of 2, 4, or 6 days, preferably 1, 2, 3 or 4 weeks, more preferably 2, 4, 6, 8, 10 or 12 months. The extremely slow flow may be measured by inverting a receptacle containing the highly viscous liquid and leaving at room temperature until the highly viscous liquid is observed to flow. In a preferred embodiment, the highly viscous liquid will not appear to flow after 2, 4 or 6 days, preferably 1, 2, 3, or 4 weeks, more preferably 2, 4, 6, 8, 10 or 12 months in an inverted position. Preferably the highly viscous liquid has a clear, transparent appearance.

Please replace paragraphs 3 and 4, on page 6, beginning on line 6, with the following amended paragraphs:

Any ~~stabilising~~ stabilizing agent is suitable for use in the first step of this invention. Suitable materials include, but are not limited to, all polyols, including carbohydrate and non-carbohydrate polyols. Preferably the ~~stabilising~~ stabilizing polyol enables the active agent to be stored without substantial loss of activity by denaturation, aggregation or other means. Particularly suitable materials include sugars, sugar alcohols and carbohydrate derivatives. Preferably, the glass forming polyol is a carbohydrate or derivatives thereof, including glucose, maltulose, iso-maltulose, lactulose, sucrose, maltose, lactose, iso-maltose, maltitol, lactitol, palatinit, trehalose, raffinose, stachyose, melezitose or dextran, most preferably trehalose, sucrose, sorbitol, raffinose, mannitol, lactose, lactitol or palatinit, most preferably sucrose, sorbitol, lactose or trehalose.

Bacterial polysaccharides are particularly advantageous for use as a ~~stabilising~~ stabilizing agent in an immunogenic composition since they can act both as a ~~stabilising~~ stabilizing agent and an immunogen.

Please replace paragraphs 3 and 4, on page 7, beginning at line 14, with the following amended paragraphs:

Amino acids can act as ~~stabilising~~ stabilizing agents and can be used by themselves and preferably in combination with a polyol. Preferred amino acids include glycine, alanine, arginine, lysine and glutamine although any amino acid, or a combination of amino acids, peptide, ~~hydrolysed~~ hydrolyzed protein or protein such as serum albumin can act as a ~~stabilising~~ stabilizing agent.

The concentration of the ~~stabilising~~ stabilizing agent used in the process of the invention may be between 1% and 50% weight/volume, preferably 1-5%, 5-10%, 5-10%, 15-20%, 20-25% or 25-50%, most preferably less than or equal to15% or 10% (w/v). The amounts of ~~stabilising~~ stabilizing agent required is proportional to the amount of salts present. Therefore, although levels of ~~stabilising~~ stabilizing agent between 2% and 10% are preferred, higher concentrations of 10% to 25% may be required to dry samples with a high salt (over 100mM, 200mM, 300mM, 400mM or 500mM) content.

Please replace paragraph 2, page 8, beginning at line 13, with the following amended paragraph:

The process of the invention preferably uses containers with a solvent repellent, preferably a water repellent interior surface. This is achieved through coating the interior surface with a hydrophobic composition, for instance by ~~siliconisation~~ siliconization. ~~Siliconisation~~ Siliconization is achieved by processes that are well known to those skilled in the art. In one method, the container is ~~siliconised~~ siliconized by ~~rising~~ rinsing the interior of the container with an emulsion of silicone, followed by processing through an oven at high temperature, typically 350 °C. Alternatively, the water repellent interior surface is achieved by the container being made of a water repellent composition.

Please replace paragraph 1, on page 9, beginning at line 1, with the following amended paragraph:

The solvent into which the ~~stabilising~~ stabilizing agent and active agent are mixed can be aqueous, organic, or a mixture of both. Sufficient aqueous solvent to dissolve the glass matrix-forming material and sufficient organic solvent to dissolve a hydrophobic substance may be used, allowing the formation of glass incorporating hydrophobic substance(s).

Please replace paragraph 3, on page 9, beginning at line 18, with the following amended paragraph:

The volume of solvent can vary and will depend upon the glass matrix-forming material and the substance to be incorporated as well as any additives. The minimum volume required is an amount necessary to ~~solubilise~~ solubilize the various components. However, homogeneously dispersed suspensions of the substance(s) can also be used. Suitable amounts of the components in specific embodiments are easily determinable by those skilled in the art in light of the examples provided herein.

Please replace paragraph 3, on page 10, beginning at line 17, with the following amended paragraph:

It is advantageous to incorporate a ~~coloured~~ colored dye into the preservation sample in order to allow easier ~~visualisation~~ visualization of the dried product of the method of the invention. This is particularly important during reconstitution to ensure that the highly viscous liquid is thoroughly reconstituted prior to use. Preferably, the ~~coloured~~ colored dye maintains its ~~colour~~ color at a neutral pH and is compatible with injection into a patient. Most preferably the ~~coloured~~ colored dye is phenol red.

Please replace paragraphs 1 and 2, on page 12, with the following amended paragraphs:

A subsequent stage of the method of the invention involves removing solvent until the preservation sample dries to form a highly viscous liquid. The sample neither freezes nor bubbles to form a foam during the secondary drying phase.
A highly viscous liquid is defined as a material with a solvent content less than or equal to 15, 12, 10, more preferably 8, 5, 4, 3, 2 or 1% (w/w) preferably measure using a Karl Fischer coulometric moisture ~~analyser~~ analyzer. The highly viscous liquid has a sufficiently low solvent content such that the active agent is preserved in a stable state for at least 3,6, 9,12 or 24 months at 4 °C, allowing the active agent to retain at least 40, 50, 60, preferably 70, 80, 90, 95% of its activity and/or antigenicity and/or immunogenicity over this period. Preferably, the highly viscous liquid has a solid, and/or clear appearance but is a glass and is able to flow very slowly over a period of 2, 4, or 6 days, preferably 2, 3 or 4 weeks, more preferably 2, 4, 6, 8, 10 or 12 months. The extremely slow flow may be measured by inverting a receptacle containing the highly viscous liquid and leaving at room temperature until the highly viscous liquid is observed to flow. In a preferred embodiment, the highly viscous liquid will not appear to flow after 2, 4 or 6 days, preferably 2, 3 or 4 weeks, more preferably 2, 4, 6, 8, 10 or 12 months in an inverted position.

In one embodiment of the invention, this is achieved by maintaining the pressure and temperature conditions at those applied in the first evaporative drying stage. For instance, the pressure is maintained at or below at or below 30, 25, 20, preferably15, 12, most preferably 10, 8 , 7, 6, 5, 4, 3, 2 or 1 mbar, while maintaining the temperature setting at a temperature above 0 °C, preferably of between 5 °C to 37 °C , 5 °C to 10 °C , 10 °C to 15 °C; 15 °C to 20 °C; 20 °C to 25 °C; 25 °C to 30 °C; or 30 °C to 37 °C. For a temperature setting of 15 °C , a pressure of 5-10mBars, preferably 6-9mBars, most preferably around 8mBars is maintained for between 4-24 hours, preferably 1-4 , 4-8, 8-12 or 12-16 hours. These temperature and pressure conditions are maintained for 1, 2, 3, 4, 5, 6, 8, 10, 12, 18 hours or more in order to obtain a highly viscous liquid with a solvent content less than or equal to 15, 12, preferably 10, 8, 5, 4, 3, 2 or 1% (w/w) preferably measured by a Karl Fischer coulometric moisture ~~analyser~~ analyzer.[[.]]

Please replace paragraphs 1 and 2, on page 13, with the following amended paragraphs:

Another embodiment of the invention increases the temperature setting during solvent removal to a higher temperature setting than that maintained earlier in the process. This allows the solvent to leave the sample at a quicker rate so that the method of the invention can be completed in a shorter time. For instance, the temperature setting is increased to above 0 °C, more preferably above 20 °C, preferably between 5 °C and 37 °C , 5 °C and 10 °C , 10 °C and 20 °C; 20 °C and 30 °C; more preferably 30 °C and 40 °C; more preferably 40 °C and 50 °C; most preferably 50 °C and 60 °C while maintaining the pressure at or below 30, 25, 20, preferably 15, 12, most preferably 10, 8 , 7, 6, 5, 4, 3, 2 or 1 mbar. These temperature and pressure conditions are maintained for at least 1, 2, 3, 4, 5, 6, 8, 10, 12 or 18 hours or more in order to obtain a solid with solvent content less than or equal to15, 12, 10, 8, 5, 4, 3, 2 or 1% (w/w) preferably measured by a Karl Fischer coulometric moisture ~~analyser~~ analyzer. This embodiment requires the active agent to be heat stable at the temperature used for the method to be carried out successfully.

A preferred embodiment of the invention reduces the pressure setting during solvent removal (step c) to a lower pressure setting than that used earlier in the process (step b). This allows the solvent to leave the sample at a quicker rate so that the method of the invention can be completed in a shorter time. It also enables a higher proportion of the solvent to be lost. For instance, the pressure setting is set to at or below 7, 6, preferably 5, 4, 3, more preferably 2, 1.5, 1, most preferably 0.8, 0.5, 0.2, 0.1 , 0.05 , 0.02, 0.01, or 0.005mbar, while maintaining the temperature at or above 0 °C, preferably between 10 °C and 20 °C; 20 °C and 30 °C; 30 °C and 35 °C or above 40 °C. These temperature and pressure conditions are maintained for 1, 2, 3, 4, 5, 6, 8, 10, 12 or 18 hours or more in order to obtain a solid with a solvent content less than or equal to 15, 12, preferably 10, 8, 5, 4, 3, 2 or 1% (w/w) preferably as determined by Karl Fischer coulometric moisture analyser analyzer (Eur. J. Pharm. Biopharm. (2000) 50; 277-284).

Please replace paragraph 3, on page 14, beginning at line 16, with the following amended paragraph:

Examples of active agents that can be preserved using a method of the invention include any bioactive substances such as pharmaceutically effective substances, including , but not limited to, antiinflammatory drugs, analgesics, ~~tranquillisers~~ tranquillizers, antianxiety drugs, antispasmodics, antidepressants, antipsychotics, ~~tranquillisers~~ tranquillizers, antianxiety drugs, narcotic antagonists, antiparkinsonism agents, cholinergic agonists, chemotherapeutic drugs, immunosuppressive agents, antiviral agents, antimicrobial agents, appetite suppressants, anticholinergics, antimetrics, antihistaminics, antimigraine agents, coronary, cerebal or ~~peropheral~~ peripheral vasodilators, hormonal agents, contraceptives, antithrombotic agents, diueretics, antihypertensive agents, cardiovascular drugs, opioids, and the like.

Please replace paragraph 3, on page 18, beginning at line 21, with the following amended paragraph:

The above ~~particularised~~ particularized active agents may also comprise one or more pneumococcal capsular polysaccharides as described below.

Please replace paragraph 1, on page 19, beginning at line 3, with the following amended paragraph:

The preferred combinations, dried by the process of the invention may be combined with other antigens in a combination vaccine which is desiccated or is preferably a liquid formulation which can be used to reconstitute the dried components. Preferred antigens to be combined with the active agents in the paragraph above include one or more of diphtheria toxoid, tetanus toxoid, whole cell pertussis (Pw), acellular pertussis (Pa) (as described below), Hepatitis B surface antigen, Hepatitis A virus, ~~Haemophilus influenzae~~ *Haemophilus influenzae* b polysaccharides, neisserial polysaccharides, ~~N. meningitidis~~ *N. meningitidis* serotype B proteins, pneumococcal polysaccharides, pneumococcal proteins or any of the antigens listed below. Bacterial polysaccharides may be conjugated to a carrier protein such as tetanus toxoid, tetanus toxoid fragment C, diphtheria toxoid, CRM197, pneumolysin, Protein D (US6342224) as described below.

Please replace paragraph 4, on page 20, beginning at line 18, with the following amended paragraph:

The polysaccharide conjugate may be prepared by any known coupling technique. For example the polysaccharide can be coupled via a thioether linkage. This conjugation method relies on activation of the polysaccharide with 1-cyano-4-dimethylamino pyridinium tetrafluoroborate (CDAP) to form a cyanate ester. The activated polysaccharide may thus be coupled directly or via a spacer group to an amino group on the carrier protein. Preferably, the cyanate ester is coupled with hexane diamine and the amino-~~derivatised~~ derivatized polysaccharide is conjugated to the carrier protein using heteroligation chemistry involving the formation of the thioether linkage. Such conjugates are described in PCT published application WO93/15760 Uniformed Services University.

Please replace paragraph 1, on page 21, beginning at line 1, with the following amended paragraph:

A further method involves the coupling of a cyanogen bromide activated polysaccharide ~~derivatised~~ derivatized with adipic acid hydrazide (ADH) to the protein carrier by Carbodiimide condensation (Chu C. et al Infect. Immunity, 1983 245 256).

Please replace paragraph 3, on page 25, beginning at line 12, with the following amended paragraph:

Highly viscous liquid of the invention may contain any of the active agents described above. The active agent preserved by the highly viscous liquid may comprise a biological system, for instance cells, subcellular compositions, bacteria, outer membrane vesicle preparations and viruses. It may alternatively or further ~~comprise a molecules~~ comprise molecules, for example proteins, peptides, amino acids, polynucleic acids, oligonucleotides, polysaccharides, oligosaccharides, polysaccharide – protein conjugates, oligosaccharide-protein conjugates. It may also comprise combinations of ~~comprising~~ two or more of the above active agents.

Please replace paragraph 1, on page 28, beginning at line 3, with the following amended paragraph:

The examples below are carried ~~our~~ out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples are illustrative, but do not limit the invention.

Please replace paragraph 2, on page 29, beginning at line 7, with the following amended paragraph:

Preservation samples containing 5%, 10%, 15% and 25% sucrose were made and added to vials. Samples were put into a freeze dryer at a temperature setting of 15 °C throughout the process. The pressure was initially reduced to 200mBars and maintained at this level for 10 minutes to allow degassing before reducing the pressure further. The pressure was further reduced to 8mbars for two to three hours during which time thermocouples inside the samples showed that the sample temperature reduced to 4 °C due to evaporative cooling. After 2-3 hours, the temperature of the samples returned to 15 °C, indicating that evaporation under these temperature and pressure conditions was near completion. During this stage of the process, the sample did not boil to form a foam or freeze so that an active agent within the sample is exposed to as little stress as possible. The sample ~~have~~ has the appearance of viscous liquid.

Please replace paragraph 3, on page 29, beginning at line 20, with the following amended paragraph:

Further drying of the samples was achieved by reducing the pressure further to 0.1mbars while keeping the shelf temperature setting at 15 °C. These conditions were maintained for a further 10-16 hours. During this phase, the sample temperature remained at 15 °C since the rate of evaporation was slow. Further drying took place and the resultant sample had a solid appearance. If the sample was ~~place~~ placed on its side, the sample contents slowed very slowly, over a period of days showing that the sample is a liquid glass of high viscosity. Figure 1 shows the appearance of the high viscosity liquid.

Please replace paragraph 4, on page 30, beginning at line 13, with the following amended paragraph:

Three separate experiments were performed in which IPV was resuspended in an aqueous solution with 10% sucrose or 10% trehalose as the ~~stabilising~~ stabilizing agent. The samples were put into ~~siliconised~~ siliconized vials which were placed into a Heto Drywinner 8-85 freeze-dryer and the temperature was set to 15 °C. The pressure was initially reduced to 35mBars to degas the sample. After 10 minutes, the pressure was further reduced to 8mBars and was kept at this level for two hours. During this period the temperature setting was kept at 15 °C and the temperature into the sample was monitored. As water evaporated from the sample, the temperature dropped to 4 °C but towards the end of the two hours, the temperature returned to 15 °C as the rate of evaporation slowed. No bubbling or foam formation occurred under these conditions. The pressure was then reduced further to 0.1mbars and these conditions were maintained for 16 hours more in the first two experiments and for 10 hours more in the third experiment.

Please replace paragraph 4, on page 31, beginning at line 10, with the following amended paragraph:

These levels of type 3 IPV antigen retention ~~compares~~ compare very ~~favourably~~ favorably with the freeze drying results shown below where very low values were usually found in the same ELISA format when a monoclonal antibody against type 3 was used.

Please replace paragraph 1, on page 33, beginning at line 1, with the following amended paragraph:

Results are shown in ~~table~~ Table 5 that contains:- a) the number of ~~responant~~ respondant rats for each IPV dilution, b) the ED50 which is the dose that is required to ensure that 50% of the rats seroconvert as assessed by the immunoprecipitation assay and c) the relative potency of the dried and reconstituted IPV compared to the undried reference IPV.

Please replace paragraph 7, on page 33, beginning at line 29, with the following amended paragraph:

Example 6 Effect of drying to form a highly viscose liquid using sucrose or trehalose as ~~stabilising~~ stabilizing agent on the ability of IPV to elicit an immunoprecipitating immune response in vivo

Please replace paragraph 2, on page 34, beginning at line 6, with the following amended paragraph:

After 21 days, sera were collected from all rats and an ~~immunoneutralisation~~ immunoneutralization assay, as described in Example 5 was used to assess the amount of ~~immunoneutralising~~ immunoneutralizing antibody that had been raised against each of Type 1, Type 2 and Type 3 polio virus.

Please replace paragraphs 1 and 2, on page 35, beginning at line 1, with the following amended paragraphs:

The amount of water remaining in samples was lower when sucrose was used as ~~stabilising~~ stabilizing agent with approximately 5% humidity remaining compared to approximately 10% when trehalose was used as the ~~stabilising~~ stabilizing agent measured by a Karl Fischer coulometric moisture ~~analyser~~ analyzer.

Both sucrose and trehalose were effective at ~~stabilising~~ stabilizing IPV during the drying process so that the reconstituted IPV gave relative potency readings approaching 1.0 for most of the different types of polio virus. The relative potencies were particularly good for Type 3 polio virus which ~~looses~~ loses its immunogenicity relatively easily.

Please replace the Abstract with the following amended abstract.

The present invention relates to a method of drying biological and other labile samples so that they can be preserved as a highly viscous liquid. The method involves the steps of

preparing a preservation sample by dissolving/suspending an active agent in a solution of a ~~stabilising~~stabilizing agent, subjecting the preservation sample to such temperature and pressure conditions that the preservation sample ~~looses~~loses solvent by evaporation without freezing or bubbling to form a foam and removing solvent until the preservation sample dries to form a highly viscous liquid.